EXHIBIT 3

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Mining the genome for combination therapies

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The evaluation of drug combinations for cancer treatment has progressed slowly through methodical clinical research. A new study examining gene expression profiles could signal a shift in the approach to combination therapy.

Effective treatment for cancer requires not only the discovery of individual drugs with antitumor activity, but also knowledge of the best way to combine these drugs. Nowhere is this more evident than in the treatment of childhood acute lymphoblastic leukemia (ALL), a disease that 25 years ago claimed the lives of the majority of ALL patients, but today is up to 90% curable[1, 2]. Over that 25-year period, no new drugs have entered standard treatment protocols; rather, it has been the optimization of combinations of old drugs, based entirely on clinical empiricism and trial and error, that has yielded such effective results. A molecular basis for such combination therapy has been lacking.

A new report by Cheok *et al.* in the May issue of *Nature Genetics* provides some of the first molecular in-sights into combination leukemia treatment[3]. Cheok *et al.* examined the DNA microarray–based gene expression profiles of childhood ALL bone marrow cells 24 hours after the randomized initiation of patient treatment with the purine antagonist mercaptopurine, the dihydrofolate reductase inhibitor methotrexate, or a combination of the two agents. The study

is unusual in that it addresses changes in gene expression on a genome-wide scale in tumor cells after short-term treatment *in vivo*.

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The first question was whether mercaptopurine and methotrexate elicit similar changes in gene expression. One might expect that because both drugs affect purine metabolism (required for nucleotide biosynthesis), the gene expression consequences of the two drugs would be similar. To the contrary, Cheok *et al.* report that the effects of mercaptopurine and methotrexate, when given as single agents, were largely non-overlapping. This result suggests that these are molecular targets of these drugs beyond those implicated in purine biosynthesis. Importantly, the authors performed these expression-profiling experiments before the onset of tumor cell death, at which point a presumably common program of gene expression would be observed.

The next question was whether the combination of mercaptopurine and methotrexate treatment resulted in gene expression changes that simply reflected the sum of the two parts. Strikingly, Cheok *et al.* report that only 14% of the genes regulated by either mercaptopurine or methotrexate alone were similarly regulated by the combination. Moreover, of over 200 genes regulated by the combination, less than 20% were regulated by the individual component drugs.

These data support a model in which the combination does not simply function as a sum of the component parts. Whether this combination treatment represents synergistic action impinging on a single molecular pathway, or rather modulation of independent pathways that subsequently converge (Fig. 1), remains to be determined.

Unfortunately, clinical efficacy data from the randomized clinical trial studied at the molecular level by Cheok *et al.* are not yet available. Correlates of molecular synergy (or antagonism) with therapeutic synergy have the potential to provide valuable insights into pharmacologic mechanisms of action, and also to guide the design of future combination clinical trials.

The molecular synergy observed by Cheok *et al.*, however, might reflect off-target, toxicity-promoting effects or molecular antagonistic effects, both of which might not translate into increased efficacy of the combination. The elucidation of efficacy-correlated molecular synergy would indeed represent a possible early molecular endpoint whose measurement in the clinical trial setting could be invaluable.

Curiously, Cheok *et al.* report that the molecular consequences of *in vivo* combination treatment were not recapitulated in cultured leukemia cell lines. They interpret this to mean that such pharmacologic interactions must be studied *in vivo*, in the setting of a clinical trial. This may be the case, but it is also possible that the result reflects analysis of only a limited number of cell lines. If such discrepancies hold up upon further analysis, however, they would add to mounting evidence highlighting the differences between cancer cell lines and the primary tumors from which they are derived.

The results of Cheok *et al.* indicate that a molecular signature of combination therapy exists, and suggest that systematic approaches to searching for such synergistic drug combinations might be feasible. Concerns over cell lines notwithstanding, one could imagine screening combinations of compounds for molecular synergy, thereby identifying combinations whose components were previously unsuspected.

The challenge, of course, will be to relate such *in vitro* molecular synergy to clinical synergy *in vivo*. Although such clinical studies would not be trivial, the past 25 years' experience in childhood ALL combination therapy is a case in point that the optimization of combinations based solely on clinical empiricism is excruciatingly slow. Certainly there is room for improvement with molecular approaches to the problem.

What is the future of combination therapy for cancer? One could argue that as mechanisms of cancer pathogenesis are elucidated, molecularly-targeted single agent therapy could gain a strong foothold in the clinic. It is more likely, however, that combination approaches will remain critical—either simultaneous targeting of a single pathway so as to avoid drug resistance, or the targeting of two or more different pathways, each of which is essential for tumor cell survival.

Early clinical experience with the tyrosine kinase inhibitor imatinib (Gleevec), targeting the *BCR-ABL* tyrosine kinase oncogene in chronic myeloid leukemia, indicates that such monotherapy can foster the emergence of drug-resistant clones[4]. Thus, the task at hand is to determine how best to combine imatinib with other agents in order to avoid such resistance. It seems likely that other kinase inhibitors will also require combination approaches to effect long-term cures.

The study by Cheok *et al.* provides another piece of

compelling evidence that the era of performing clinical trials without molecular studies is coming to an end. Sophisticated molecular surveillance is becoming increasingly embedded in clinical trial design in an effort to shorten the time and lessen the cost of clinical trials. It is now clear that the collection of such genomic information as part of clinical trials is feasible; the extent to which this information will be truly useful is less certain.

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